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MECHANIC ARTS

Doctoral Thesis No. 96

STUDIES ON THE  
INHERITANCE OF RESISTANCE TO THE DANYSZ  
BACILLUS IN THE RAT

A DISSERTATION  
SUBMITTED TO THE GRADUATE FACULTY  
IN CANDIDACY FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

BY

M. R. IRWIN

Reprinted from GENETICS 14: 337-365, July, 1929

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# THE INHERITANCE OF RESISTANCE TO THE DANYSZ BACILLUS IN THE RAT<sup>1</sup>

M. R. IRWIN

*Bussey Institution, Harvard University, Forest Hills, Boston, Massachusetts*

Received November 12, 1928

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## INTRODUCTION

Differences between species and genera in their resistance to certain bacterial diseases, such as the immunity of man to many diseases of the lower animals, are commonly accepted as being due to a natural immunity. Less common are differences in resistance between races within the species, as the generally accepted superiority of resistance to tuberculosis of the white race to the negro in America. The higher resistance to anthrax attributed to the Algerian sheep as compared to that of other races of sheep is another example of the same type of relative immunity.

Individual differences in resistance are well known phenomena, nowhere better illustrated than in natural epidemics among laboratory animals. Observations during such epidemics, in which stocks of mice differing in hereditary factors differed also in resistance to the epidemics, have been reported by TYZZER (1917) and by HAGEDOORN-LABRAND and HAGEDOORN (1920). In each case reported the Japanese waltzing mouse stocks were much less resistant to the different infections than were the other mouse stocks.

More critical evidence of the role of heredity in resistance has been presented by WEBSTER (1924-25), by ROBERTS and CARD (1926), and by

<sup>1</sup> Paper No. 23 from the Department of Genetics, IOWA STATE COLLEGE, Ames, Iowa.

LAMBERT and KNOX (1928). WEBSTER demonstrated that the resistance of a strain of mice to a fixed dose of a specific organism could be enhanced or diminished by selective breeding. The experiments of ROBERTS and CARD on bacillary white diarrhea of chicks showed that resistance to this disease is determined in part by heredity. LAMBERT and KNOX, using controlled doses of the organism on baby chicks, suggested multiple genetic factors as in part determining the resistance to fowl typhoid in chickens.

WRIGHT and LEWIS (1921) found distinct differences in resistance to tuberculosis among highly inbred lines of guinea-pigs. PRITCHETT (1926) asserted clear-cut differences between inbred strains of mice in their susceptibility to controlled mouse typhoid infection. FRATEUR (1924) proposed a single factor difference between resistance and susceptibility of fowls to avian diptheria.

The object at the outset of the investigations reported in this paper was to determine the part played by heredity in resistance to a specific disease. The rat was chosen as the animal to be subjected to the tests because of its well known prolificacy and general hardiness. The organism used has shown itself after repeated tests to possess a much higher degree of pathogenicity for the rat as host than many other organisms belonging to the same general group.

The data presented by the author (1928) in a preliminary report of this experiment are included in this paper.

#### MATERIALS AND METHODS

A stock of rats (*Rattus norvegicus*), pen inbred for about eight years, has been the source of a part of the animals used in these investigations. Descendants of a pair of the inbred Wistar "A" strain, obtained in 1924 from Doctor HELEN DEAN KING of the Wistar Institute, constituted the other source. The environmental factors, care, feeding, and housing of these animals have been kept as constant as possible throughout the experiment.

A culture of the Danysz bacillus was supplied in 1926 through the courtesy of Doctor Rob W. SPRAY, of WEST VIRGINIA UNIVERSITY. The organism had been isolated by him from a commercial rat virus, and cultured on plain agar slants. In our laboratory, it has been maintained by monthly cultures on veal infusion agar slants.

This organism belongs to the paratyphoid-enteritidis group, differing from some other members of the group by exhibiting much more pathogenicity for the rat when injected intraperitoneally. Exact cultural and

serological relationships between this organism and other members of the group have not been completely determined to date; hence the name under which it was received has been retained.

The media, temperature, and incubation period of the bacterial culture have been kept constant. For an injection period, a suspension of each 18 hour culture was made in 4 cc of sterile physiological salt solution and mixed in a sterile serum bottle. Since the organism is motile, it was necessary to attenuate a small sample of the total suspension by heating at 55-60° C. for 20 minutes. Counts were then made from the attenuated sample on a Max Levy counting chamber. After the number of organisms per cc was determined, the original suspension was diluted so that the required inoculum was contained in 0.25 cc.

Each animal was injected intraperitoneally with a specific dose. The average age at injection of the animals was approximately 50 days. The deaths for the preceding 24 hours were recorded each morning at about 10 o'clock. In general, only those deaths were recorded which occurred between the 2nd and 14th days after injection, inclusively.

Preliminary tests have been made to determine the viability of the organism in saline solution. Were there a great difference in the number of bacteria present in the suspension after the lapse of an hour or more, it would follow that differences in percentage mortality between those animals injected at the beginning and at the end of the injection period might be noted. However, a few counts made from five to seven hours after the organism had been put in suspension gave no indication of any appreciable increase or decrease in number during that interval of time. Further, sets of animals inoculated after such a lapse of time have given the same mortality in general as those inoculated several hours previously.

However, in this experiment, the time between making the suspension, counting the organisms, and injecting the animals has been relatively constant. Any change, then, in the number of organisms in the suspension has been in a constant relative ratio to the number injected.

#### PERCENTAGE MORTALITIES DUE TO VARYING NUMBERS OF BACTERIA

A very important consideration in an experiment of this nature is that the virulence of the organism injected at the various times should remain uniform. With a constant virulence, the percentage of deaths following injection of a specific organism, would vary largely with the number of organisms and with the degree of resistance of the host.

To test for uniformity of virulence of the organism, and to determine the number that would cause the death of the majority of the animals

injected, graded series of doses were given at various intervals. The mean mortalities resulting from the injections of these different doses are shown in table 1, with their probable errors expressed in percent. The probable errors are given primarily as an indication of the ranges of the means, and not as a basis of comparison.

It may be noted that a decrease in the number of organisms injected was accompanied by a decrease in average mortality. The two massive doses, 2000 to 4000 million organisms respectively, offer very good indications that it is possible to inject a sufficient number of organisms to kill any individual. The one survivor of the heavier of these two doses succumbed within six days after the final day following injection on which the mortality records were recorded.

TABLE I

*Percentage mortalities in a pen inbred stock of rats due to the injection of different numbers of the organism.*

| DOSE<br>(MILLIONS) | NUMBER<br>INJECTED | NUMBER<br>DEAD | PERCENTAGE<br>MORTALITY |
|--------------------|--------------------|----------------|-------------------------|
| 4000               | 80                 | 79             | $98.7 \pm 1.1$          |
| 2-4000             | 39                 | 39             | 100.0                   |
| 275                | 134                | 127            | $94.7 \pm 1.7$          |
| 210                | 93                 | 85             | $91.3 \pm 2.4$          |
| 150                | 431                | 365            | $84.7 \pm 1.3$          |
| 120                | 102                | 66             | $64.7 \pm 2.6$          |

If 150 million organisms are taken as a basis of comparison, it may be noted that a decrease of 30 million from this number was accompanied by a much greater difference in mortality percent (20) than for an increase of 60 million organisms (6.6). Further increases in numbers over this dose were not accompanied by marked increases in the mortality. For this reason, 150 million organisms were chosen as the minimum for a "standard" dose, and have so been used in the course of this experiment.

The death rate by days for these different doses is given in figure 1. These curves are based on percentage mortalities up to and including the 14th day after inoculation.

A comparison of the curves shows that the subjects given a very massive dose tended to die very rapidly directly following the injection; the few individuals remaining then showed a somewhat decreased death rate. This would indicate a very acute form of the disease, while those animals surviving the acute form seemingly suffered a more chronic or sub-acute infection until death or recovery.

The decreases in dose reveal a lessening of the acute type of infection, and a consequent increase in those subjects with a sub-acute type. Particularly is this noticeable in the results accompanying the injection of the smallest dose, 120 million organisms, for which we note a very gradual rate of death until the ninth day, in contrast with the heavier doses in which the majority of the deaths occurred before the seventh day following the injection.

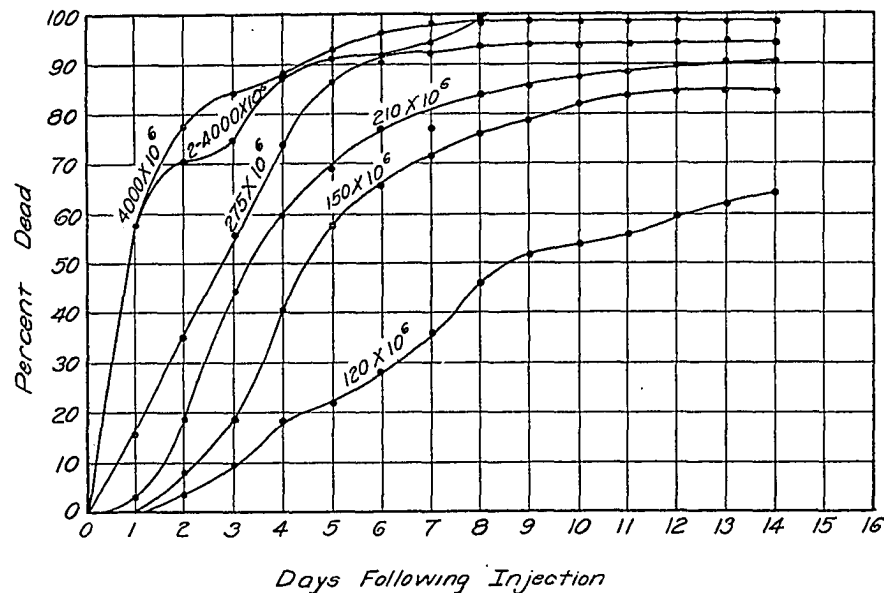


FIGURE 1.—Death rates showing effects of varying doses of the organism.

There was considerable difference in the rate of death due to the injection of 120 million when compared with 150 million organisms; increases in number above the "standard dose" showed no such appreciable difference in the rate of mortality. Thus both the percentage mortality and the rate of mortality in this stock tend to indicate marked changes for a dose less than 150 million.

A statistical analysis of the observed differences between the various doses is given in table 2. The calculations have been made according to FISHER'S (1925, p. 84) contingency table, which tests the independence of the proportionate differences between the two samples being compared.

In this table the two very heavy doses of two and four billion organisms have been eliminated from the comparison, and only the differences between the other doses have been calculated. The chief point of interest in the table is, that by taking 150 million organisms as the standard, a



decrease in the injected dose of 30 million was accompanied by a significant decrease in the proportionate number of animals killed, while an increase of 60 million organisms gave only a slight approach to a significant difference. An injection of nearly twice this standard number was necessary to produce a significant increase in mortality. It would seem necessary then, in order to establish significant differences in mortality between a dose of 150 million organisms and a somewhat greater number, to use larger numbers of test animals than would be possible under ordinary laboratory conditions. The trend in percentage mortalities, however, due to the influence of varying numbers of bacteria injected is unmistakable, and shows the results to be expected by using different numbers of these bacteria.

TABLE 2

*Analysis of differences in percentage mortalities due to varying doses of the organism, as given in table 1.*

| DOSES (MILLIONS)<br>COMPARED | DIFFERENCES IN<br>PERCENTAGE<br>MORTALITY | $\chi^2$ | P     |
|------------------------------|---|----------|-------|
| 275 and 210                  | 3.4                                       | 1.068    | 0.30  |
| 275 and 150                  | 10.0                                      | 9.210    | <0.01 |
| 275 and 120                  | 30.0                                      | 35.078   | <0.01 |
| 210 and 150                  | 6.6                                       | 2.904    | 0.09  |
| 210 and 120                  | 26.6                                      | 19.881   | <0.01 |
| 150 and 120                  | 20.0                                      | 21.710   | <0.01 |

Having established that the number of bacteria injected played a considerable part in the number of deaths resulting from the induced infection, the next step is to examine the data to determine the constancy of the virulence of the organism and the uniformity in the reaction of the host. If either of these variables has not maintained a reasonable constancy, our measurement of the effect of different numbers of the bacteria has been in vain. Given a constant number of organisms, have the percentage mortalities from successive inoculations over a considerable period of time shown fluctuations which could be attributed to a change in virulence, or to the random selection of the host? Obviously, were the virulence of the organism to change markedly, no critical analysis either of the effect of the number of the organisms injected, or of the uniformity in reaction of the host would be possible.

The usual method in genetical work of calculating distributions in a series of observations, in which  $p$  represents the probability for a certain event, and  $q$  represents the difference between the value of that probability ( $p$ ) and 1, ( $q=1-p$ ), presupposes that a constant probability

underlies the frequency ratios obtained. If this underlying probability were not constant, as is often the case in statistical work, some means of testing for the type of distribution present is necessary.

The Lexis theory, as presented by A. FISHER (1922, Chapters X and XII) and by RIETZ (1927, Chapter VI), offers a method of analyzing a set of observations involving probabilities to determine the type of its distribution. In brief the method of LEXIS is to compare the dispersion of a set of observations dealing with success or failure of a certain happening with the dispersion of a Bernoulli (normal) series as a standard. The dispersion in a Poisson series is less than, while that of a Lexis series is greater than, the dispersion of a Bernoulli series.

Since the exact values of the underlying probabilities for these sets are unknown, the weighted mean and its difference from unity provide the best substitute in each set for  $p$  and  $q$ , respectively. First, the standard deviation ( $\sigma$ ) of the set of observations is calculated directly from the data at hand. Then, assuming that the series follows the Bernoulli distribution, the theoretical value of the standard deviation is calculated. The ratio between the calculated and the theoretical standard deviations ( $\sigma/\sigma_B$ ) is called the Lexian ratio ( $L$ ). The value of this ratio gives an idea of the nature of the statistical series.

"When  $L = 1$ , the series is called by LEXIS a normal series.

When  $L > 1$ , the series is called hypernormal.

When  $L < 1$ , the series is a subnormal series." A. FISHER (1922)

A completion of this method of analysis is proposed by R. A. FISHER (1925, p. 79). The  $L^2$  obtained from the Lexian ratio, when multiplied by  $N$ , the number of subsets or samples, is the same as the  $X^2$  of FISHER's table. The value of  $X^2$ , with the resulting value of  $P$ , gives a very good indication as to whether the deviation from normal of the probabilities within the set may or may not be attributed to errors of random sampling.

An example of the method of calculating  $L^2$  is given in the accompanying table 3, which presents the results of injecting 15 samples of rats with the standard dose of the organism. In the formulas,  $I$  represents the number injected at each particular time,  $K$  the number killed,  $p$  the percentage mortality at each time,  $p_0$  the weighted mean mortality percentage,  $q_0 = 1 - p_0$ ,  $N$  the number of trials, and  $n$  the total number injected.

$$p_0 = \frac{\sum K}{\sum I} = 0.847$$

$$q_0 = (1.0 - 0.847) = 0.153$$

The variance ( $\sigma^2$ ) was calculated directly from the data according to

the formula proposed by WALLACE and SNEDECOR (1925, p. 9), substituting K for X, and using 0 for the guess number for  $p_0$ .

TABLE 3  
The results obtained over a period of 16 months from the injection of 15 samples of a population of rats with the standard dose ( $150 \times 10^6$ ) of the organism.

| NUMBER<br>INJECTED<br>(i) | NUMBER<br>DEAD<br>(k) | PERCENTAGE<br>MORTALITY<br>(p) |
|---------------------------|-----------------------|--------------------------------|
| 40                        | 31                    | 77.5                           |
| 12                        | 10                    | 83.3                           |
| 22                        | 19                    | 86.3                           |
| 26                        | 22                    | 84.6                           |
| 43                        | 36                    | 83.7                           |
| 25                        | 21                    | 84.0                           |
| 17                        | 14                    | 82.3                           |
| 20                        | 17                    | 85.0                           |
| 11                        | 10                    | 90.9                           |
| 37                        | 35                    | 94.6                           |
| 39                        | 35                    | 89.2                           |
| 47                        | 47                    | 100.0                          |
| 29                        | 22                    | 75.8                           |
| 43                        | 31                    | 72.0                           |
| 20                        | 15                    | 75.0                           |
| 431                       | 365                   | 84.7                           |

Thus

$$\sigma^2 = \frac{\sum I p^2}{\sum I} - 10000 p_0^2 \quad (1)$$

$$\sigma^2 = 7233.7 - 7174.1 = 59.6 \text{ (per 100)}$$

$$\sigma = 7.72.$$

To secure the weighted standard deviation of the hypothetical Bernoulli series ( $\sigma_B$ ), which would allow for a different number of animals treated at the various injection periods, use was made of the formula proposed by A. FISHER (1922, p. 160) which resolves itself into:

$$\sigma_B^2 = \frac{N \times 100}{n} (p_0 \times q_0 \times 100) \quad (2)$$

$$= \frac{15 \times 100}{431} (0.847 \times 0.153 \times 100)$$

$$= 45.19 \text{ (per 100)}$$

$$\sigma_B = 6.72$$

$$L^2 = \frac{59.6}{45.19} = 1.32$$

$$L = 1.15.$$

The dispersion in this case is slightly supernormal, suggesting that there are differences among the samples injected.

To complete the analysis of dispersion according to FISHER (1925, p. 79):

$$L^2 = \frac{X^2}{N} \quad X^2 = NL^2 \quad (3)$$

$$X^2 = 15 \times 1.32 = 19.77.$$

From the  $X^2$  tables, under the values of  $X^2$  for  $n = 14$  ( $N-1$ ),

$$P = 0.14$$

Hence the dispersion of the average mortalities within this population was well within the errors of random sampling. The value for  $L$  (1.15) with the resulting value of  $P$  (0.14), denotes that the distribution of the chances of death within the set was practically of the normal<sup>1</sup> type.

We have no evidence, then, from the examination of the different injections with this standard dose, to suspect any change in virulence of the organism; rather the belief is strengthened that the differences were due to variations in host reaction. In table 4 the results of the tests for all the different numbers of organisms injected are analyzed according to the proposed method.

TABLE 4

*Test for the type of dispersion of probabilities within each of the populations of rats given different doses of the organism.*

| DOSE<br>(MILLIONS) | NUMBER<br>INJECTED | NUMBER<br>OF GROUPS | L    | $X^2$ | P    |
|--------------------|--------------------|---------------------|------|-------|------|
| 4000               | 80                 | 7                   | 1.05 | 7.67  | 0.27 |
| 275                | 134                | 5                   | 1.31 | 8.55  | 0.08 |
| 210                | 93                 | 3                   | 1.22 | 4.44  | 0.11 |
| 150                | 431                | 15                  | 1.15 | 19.77 | 0.14 |
| 120                | 102                | 4                   | 0.77 | 2.41  | 0.39 |

It will be seen that with but one exception, the samples injected with the varying doses show a slightly "supernormal" dispersion, as measured by the value of  $L$ . In no case, however, does the dispersion vary from the weighted mean to an extent not well within the possibilities arising from random sampling. The slightly supernormal dispersion for each of the first four named populations indicates that the differences are due to causes probably within the populations tested, and not to a change in the virulence of the organism.

<sup>1</sup> The term normal in this paper refers to the type of distribution in which the underlying probability is constant throughout.

The population injected with 120 million organisms shows a slightly subnormal dispersion ( $L=0.77$ ), suggestive that the differences in mortality vary from sample to sample, but that a constant probability prevails throughout. The small value of  $X^2$  for this population gives only a small probability ( $P=0.39$ ) that the discrepancies from a normal dispersion are due to causes other than random sampling.

The inference, then, has strong support that there has been no variation in mortality in the samples of the animals injected with the given doses which might not have been attributed to chance. Since the animals injected with these varying doses were all taken from the same general stock, it can be stated with reasonable assurance that the virulence of the organism has remained practically constant throughout the experiment.

The population injected with the minimum standard dose shows by this analysis that the variance was due largely to causes among the samples and presumably within the population itself. This would follow a logical line of reasoning, for if an infection with organisms of constant virulence and in approximately equal numbers would produce an average proportion of 85 deaths to 15 survivors, the chances of death from subset to subset would vary as the animals themselves would vary. Inherent differences as well as small environmental fluctuations might well increase or decrease the chance of survival of individuals within a sample.

A method has been proposed to examine a set of observations involving probability to determine the type of distribution therein. Presumably, if the distribution were essentially normal, the probable error should be an accurate estimate of the trustworthiness of the observations. If, however, we wish to compare two such sets, as of two populations of animals injected with different numbers of bacteria, it is of interest to note whether the ratio of the mean differences to the probable errors of their differences gives a correct estimate of their relationship.

A part of table 2 is repeated in table 5, and in addition, the ratios of the differences of the mean mortalities of the different doses to their probable errors are given. The probable errors of the means, using the  $\sigma$  of each set calculated directly from the data by formula (1) above, were obtained from the following formula:

$$P \cdot E_m = .67449 \frac{\sigma}{\sqrt{N}} \quad (4)$$

Since we have no satisfactory method of computing the correlation between the samples injected with different doses, the probable errors of the differences between any two doses, a and b, have been determined by the formula

$$PE_{a-b} = \left[ \frac{2}{PE_a} + \frac{2}{PE_b} \right]^{1/2}$$

No appreciable differences are noted when the two methods of comparison, Fisher's test for independence and the ratio of the mean differences to their probable errors, are used. Each method gives approximately the same probability for or against significant differences being due to the injection of the different doses compared.

TABLE 5

*A comparison of the test for independence and the probable error of the mean differences as to their reliability in analyzing differences in percentage mortalities due to varying numbers of bacteria.*

| DOSES<br>(MILLIONS)<br>COMPARED | DIFFERENCES IN<br>PERCENTAGE<br>MORTALITY | X <sup>2</sup> | P     | MEAN PERCENTAGE<br>DIFFERENCE WITH<br>PROBABLE ERROR | DIFF. |
|---------------------------------|---|----------------|-------|--|-------|
|                                 |   |                |       |  | P.E.  |
| 275 and 210                     | 3.4                                       | 1.068          | 0.30  | 3.4±2.9  | 1.2   |
| 275 and 150                     | 10.0                                      | 9.210          | <0.01 | 10.0±2.2   | 4.6   |
| 275 and 120                     | 30.0                                      | 35.078         | <0.01 | 30.0±3.1   | 9.7   |
| 210 and 150                     | 6.6                                       | 2.904          | 0.09  | 6.6±2.9  | 2.3   |
| 210 and 120                     | 26.6                                      | 19.881         | <0.01 | 26.6±3.5   | 7.6   |
| 150 and 120                     | 20.0                                      | 21.710         | <0.01 | 20.0±2.9   | 6.9   |

It is well to take into consideration, however, that in estimating the probable errors according to formula (4) that the N in the dividend is not always the same from set to set. This might possibly make a slight difference in the comparative values of the probable errors. Further, accurately to estimate the probable error of the mean differences between any two such populations, the amount of correlation existing between them should be considered.

The X<sup>2</sup> test for independence does take the correlation between the two populations under comparison into account, and may also be used when the underlying probability of chances for death is not constant. Hence it has been taken as the basis of comparison between all stocks compared in this experiment.

#### SELECTION FOR RESISTANCE

At the beginning of this experiment, it was believed that a part of the individuals surviving a highly fatal injection of organisms possessed more natural resistance to the infection than did those which succumbed. If the differences in resistance were in part due to heredity, the progeny of survivors should consist of a relatively higher proportion of individuals resistant to a similar infection than did the original stock.

It was further believed that greater differences in the degree of resistance would probably be found among individuals in the pen-inbred stock of our laboratory than among the more closely inbred animals. If experimental results established this difference, it should be possible, by selection, to produce a stock of animals much more resistant than the parental pen-inbred stock, and probably also more highly resistant to the organism than a similarly selected stock among the inbred animals.

The results of selection up to the present are summarized in table 6, giving the mean mortality for each stock. The system of naming the various stocks is as follows: The S stock represents descendants of the Wistar "A" strain which have been selected for susceptibility; Ra the random stock; R<sub>1</sub> (first resistant generation) was produced from matings of survivors of the Ra stock; R<sub>2</sub> and R<sub>3</sub> represent the second and third resistant generations, respectively. Survivors of the R<sub>1</sub> stock, mated to non-tested individuals of the S stock, produced the F<sub>1</sub> stock: survivors of this F<sub>1</sub> stock, mated to the S stock or *inter se*, produced the backcross and F<sub>2</sub> stocks, respectively.

TABLE 6

*Percentage mortalities of stocks of rats differing in hereditary composition, due to the injection of the standard dose.*

| STOCKS         | NUMBER<br>INJECTED | NUMBER<br>DEAD | PERCENTAGE<br>MORTALITY |
|----------------|--------------------|----------------|-------------------------|
| S              | 228                | 211            | 92.5                    |
| Ra             | 431                | 365            | 84.7                    |
| R <sub>1</sub> | 137                | 58             | 42.3                    |
| R <sub>2</sub> | 227                | 74             | 32.6                    |
| R <sub>3</sub> | 117                | 41             | 35.0                    |
| F <sub>1</sub> | 175                | 96             | 54.9                    |
| Backcross      | 163                | 81             | 49.7                    |
| F <sub>2</sub> | 200                | 51             | 25.5                    |

Table 7 gives the analysis of the differences between parent and progeny stocks of table 6.

TABLE 7

*Summary of tests for independence between certain of the stocks of table 6.*

| STOCKS<br>COMPARED                | DIFFERENCES IN<br>MORTALITY | $\chi^2$ | P     |
|-----------------------------------|-----------------------------|----------|-------|
| Ra and S                          | 7.8                         | 11.606   | <0.01 |
| Ra and R <sub>1</sub>             | 42.4                        | 88.470   | <0.01 |
| R <sub>1</sub> and R <sub>2</sub> | 9.7                         | 3.488    | 0.06  |
| F <sub>1</sub> and S              | 37.6                        | 77.426   | <0.01 |
| Backcross and S                   | 42.8                        | 92.152   | <0.01 |
| Backcross and F <sub>2</sub>      | 24.2                        | 22.656   | <0.01 |

The mean mortality of the S strain was somewhat higher (7.8) than that of the Ra stock. As this difference has been constant throughout the experiment, the S individuals have been termed the "susceptible" strain. The  $X^2$  test gives practical assurance that the differences between these two strains are not assignable to the errors of random sampling for the present numbers; also, the trend towards more susceptibility of the S stock has been very noticeable.

The progeny ( $R_1$ ) of the survivors of the Ra stock showed a decided increase in the proportionate number of resistant individuals over its parent stock (42.4 in 100). The high value for  $X^2$  in the comparison of the mortalities of these two strains puts the difference in proportionate deaths clearly outside the bounds of chance ( $P < 0.01$ ). We have no reason to doubt that the increased number of individuals in the  $R_1$  stock resistant to the infection was due in very large part to immunity factors transmitted by the parent survivors.

The next resistant generation likewise gave a decrease in mortality over the  $R_1$  stock, although not as marked as for the preceding generation.  $X^2$  for the numbers tested in these two generations shows an approach to significance ( $P = 0.06$ ), but much larger numbers in each stock would be necessary definitely to establish that selection was the chief cause for the difference in mortality. The  $R_3$  stock has to all practical purposes the same mean mortality as its parent stock,  $R_2$ . No appreciable effect of selection was apparent in this stock, for reasons to be suggested later.

A comparison of the death rates by days for these five stocks is given in figure 2. Stocks S and Ra have a somewhat similar death rate, the curve for S being slightly steeper throughout. Not only were a somewhat greater number of the S stock susceptible than of the Ra stock, but the time to death of the majority of the subjects in this strain was also shorter.

A very significant decrease in mortality rate is noticeable in the curve of the  $R_1$  from that of the Ra stock: the  $R_2$  stock in turn shows a slight decrease in time to death in comparison with its parental stock. No deaths occurred in the  $R_3$  stock until the third day following injection, but it is perhaps significant that the death rate for the two days following was much higher than for either of the other two stocks selected for resistance: after the fifth day, however, the curve becomes flattened. The majority of the deaths in this stock occurred then, very shortly after the day of injection. A more detailed analysis of the reaction of this stock will be taken up later.

Referring again to table 6, we note that the first hybrid generation,  $F_1$  stock, showed a decided drop in mortality from that of the susceptible



S parent (from 92.5 to 54.9 percent), beyond the errors of random sampling; and it differed to a much less extent from the mean mortality of the  $R_1$  parent stock (54.9 as compared to 42.3 percent). The resistance was transmitted to the offspring by either sex of the  $R_1$  stock as will be shown in table 9.

If we assume that the  $R_1$  stock possessed many hereditary factors with potentiality for resistance and for susceptibility as well, as evidenced in part by the small decrease in mortality of its selected progeny  $R_2$ , the  $F_1$  individuals surviving the infection were presumably those which received a high potentiality of resistance from the  $R_1$  parent. The parental S strain, being quite uniformly susceptible, would probably contribute but little in potential resistance to the offspring.

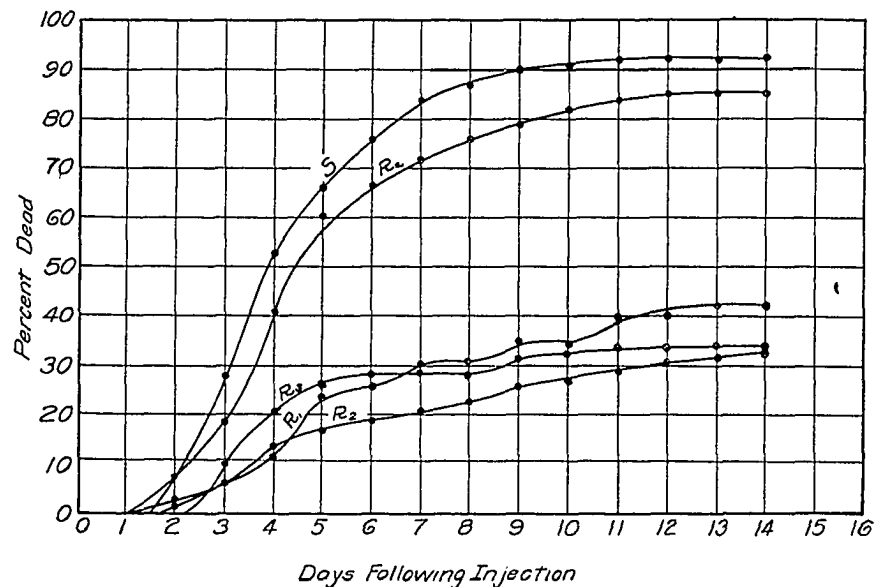


FIGURE 2.—Death rate curves of different stocks selected for resistance.

The reaction of the progeny of the matings of these hybrid survivors again to the S stock (backcrosses) and among themselves should present further evidence as to whether or not the increased resistance of the hybrid generation over the susceptible S parent was due chiefly to heredity; and also give an indication of the type of inheritance of the resistance.

It is surprising to note that the mortality of the backcrossed stock is approximately equal to that of the hybrid parent, being 49.7. It must be remembered, however, that the  $F_1$  parents were selected stock, in that

they had survived an infection, and had been chosen from lines exhibiting a high degree of resistance. The  $X^2$  test for independence between the backcrossed progeny and the susceptible parent makes certain that the differences in resistance were due to causes other than chance; practical certainty that the resistance was transmitted through the selected hybrid parent to the offspring.

The  $F_2$  generation in turn gave a decided increase in the number of resistant individuals over the backcrossed stock; for these numbers as given it showed the least mortality of any of the stocks of animals. The two types of matings,  $F_1 \times S$ , and  $F_1 \times F_1$ , indicate very strikingly in the

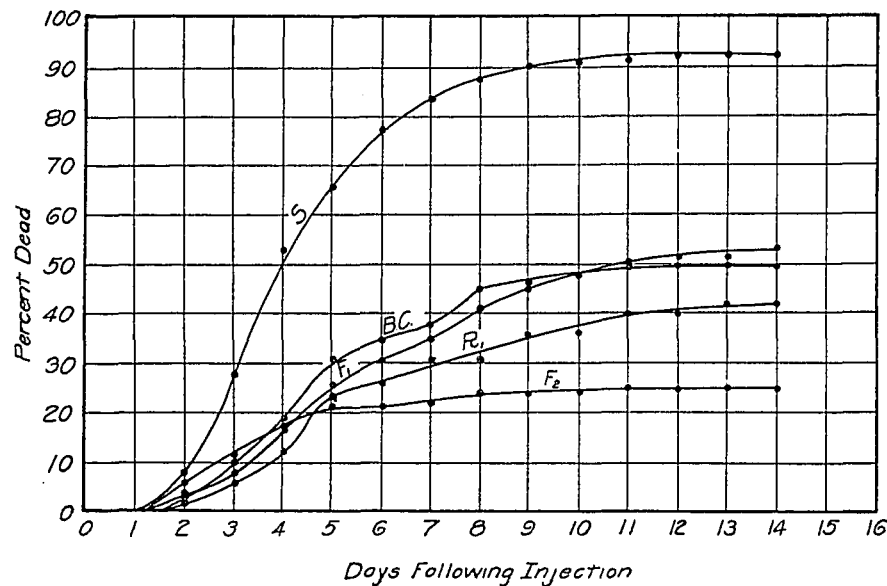


FIGURE 3.—A comparison of death rates of the hybrid stocks with those of their parent strains.

reaction of their progenies that the resistance depended in large part upon the hereditary contributions of the parents. The differences in proportionate deaths between the backcrossed and  $F_2$  progeny are too large to come within the errors of random sampling ( $P < 0.01$ ), and we have strong support for the inference that hereditary factors are largely responsible for the difference. Further, the reactions of the  $F_1$ , of the backcrossed, and of the  $F_2$  stocks to the infection gave very good indication that the hereditary resistance to this organism was produced in large part by the action of a complex of at least partially dominant factors.

If we examine the death rate curves for these various stocks as presented in figure 3, we find a very close association between the rate of

mortalities and the percentage mortalities. The difference between the rates of death of the parental strains of the hybrid stocks has already been discussed. It is interesting to observe the similarity of the curves representing the death rates for the  $F_1$  and backcross generations: each depicting a very gradual rate of death. This agrees very well with their approximate equality of mortality.

The curve for the  $F_2$  generation suggests a situation somewhat analogous to that observed for the  $R_3$  stock, in that the greater majority of the deaths for both stocks occurred during the first few days following the injection, followed by an appreciably lessened rate of death. It might well be reasoned that the individuals of the  $F_2$  stock which suffered an early death were those possessing combinations of factors for susceptibility, with relatively few factors for resistance.

Having established that stocks of rats of different genetic constitution differed in the degree of their reactions to a fixed dose of a specific organism, it is well to examine each stock to ascertain if this reaction is uniform within the various stocks. Each stock, with the exception of the Ra as noted, has been divided into its various litters as subsets, and the theory of LEXIS applied as a test for the type of distribution of the probabilities. The results are summarized in table 8.

TABLE 8  
*Test for the type of dispersion of the probabilities within each of the several stocks of table 6.*

| STOCKS    | NUMBER<br>INJECTED | NUMBER<br>OF LITTERS | L    | $\chi^2$ | P     |
|-----------|--------------------|----------------------|------|----------|-------|
| S         | 228                | 46                   | 1.49 | 101.66   | <0.01 |
| Ra        | 431                | 15 <sup>1</sup>      | 1.15 | 19.77    | 0.14  |
| $R_1$     | 137                | 23                   | 1.60 | 58.80    | <0.01 |
| $R_2$     | 227                | 33                   | 1.38 | 63.03    | <0.01 |
| $R_3$     | 117                | 18                   | 1.66 | 49.84    | <0.01 |
| $F_1$     | 175                | 32                   | 1.75 | 97.90    | <0.01 |
| Backcross | 163                | 24                   | 1.54 | 57.12    | <0.01 |
| $F_2$     | 200                | 28                   | 1.60 | 71.40    | <0.01 |

<sup>1</sup> Signifies groups rather than litters.

Due to long continued brother-sister matings, it would very naturally be supposed that an inbred stock of rats, such as the S descendants of the Wistar "A" strain, should be practically homozygous for many of its hereditary factors. In its reaction to this infection, however, an analysis of the 46 litters of this strain, gives good indication ( $P < 0.01$ ) that there were differences in resistance between litters within this strain, and consequently within certain litters, that were not ascribable to errors of random sampling.

This belief is given further support if we examine the litters themselves for possible discrepancies. By eliminating four litters from the total, (examination of the reactions of these four litters showed that they differed markedly from the average mortality) which were injected at as many different times, the following result is obtained:

| <i>Number<br/>litters</i> | <i>Number<br/>tested</i> | <i>Percentage<br/>mortality</i> | <i>L</i> | <i>X<sup>2</sup></i> | <i>P</i> |
|---------------------------|--------------------------|---------------------------------|----------|----------------------|----------|
| 46                        | 228                      | 92.5                            | 1.49     | 101.66               | <0.01    |
| 42                        | 211                      | 96.2                            | 1.37     | 78.90                | <0.01    |

Although the resulting value of P is also practical certainty that the deviations from a normal dispersion could not be ascribed to chance, it is of value to note that the elimination of the four litters increased the mortality by 3.7, and decreased the Lexian ratio. This stock will be analyzed further for causes of the discrepancies under the discussion of sex differences.

The essentially normal type of distribution of probabilities of the Ra stock has already been considered. The remaining stocks all showed a supernormal type of dispersion, since L for each is greater than 1, and in no case is the large value of X<sup>2</sup> to be attributed to chance alone. This is quite suggestive that the causes for the discrepancies are to be sought within the stocks themselves, indicating that considerable differences in resistance as measured by percentage mortality have been found between litters, as well as within the litters of each stock. It is worth mentioning here that grouping two of the stocks, S and R<sub>1</sub>, by their separate injection periods rather than by litters, gave approximately the same comparative values for L and P as shown in table 8.

The type of dispersion shown by these selected stocks lends strong support to the inference that the stocks varied in their resistance from litter to litter, and consequently no true measure of the amount of dispersion can be given, lacking *a priori* grounds for the probability of death of any individual within a litter. This agrees with the suggestion that the differences were due, in large part at least, to the hereditary factors for resistance which had a major influence in determining the chance of death or survival at inoculation.

#### PASSIVE IMMUNITY

It has been established in the preceding pages that selection may enhance the resistance of a stock of rats to a controlled infection. To test how much, if any, of this increase in number of resistant animals was due to the transmission of passive immunity, a few litters of the R<sub>1</sub> stock were not inoculated, but were mated to members of the susceptible S stock.

The first item in table 9 gives the mortality of the progeny of this particular type of mating. The progeny resulting therefrom did not differ appreciably in its reaction from that of the progeny of matings of surviving  $R_1$  individuals with members of the S stock. The slightly lower mortality for the first item when compared with that for the second (51.4 and 54.9 respectively) is non-significant.

TABLE 9  
*Summary of tests for indication of the presence of passive immunity.*

| MATINGS   | NUMBER<br>INJECTED | PERCENTAGE<br>MORTALITY | NUMBER<br>LITTERS | L    | $\chi^2$ | P     |
|---|--------------------|-------------------------|-------------------|------|----------|-------|
| F <sub>1</sub> stock                                      |                    |                         |                   |      |          |       |
| 1. $R_1$ (non-injected) $\times$ S                        | 107                | 51.4                    | 19                | 1.41 | 38.24    | <0.01 |
| 2. $R_1$ (survivors) $\times$ S                           | 175                | 54.9                    | 32                | 1.75 | 97.90    | <0.01 |
| $R_1$ $\sigma^7 \times$ S $\phi$ $\phi$                   | 85                 | 44.7                    | 20                | 1.39 | 38.80    | <0.01 |
| $R_1$ $\phi$ $\phi \times$ S $\sigma^7 \sigma^7$          | 90                 | 64.4                    | 12                | 2.18 | 57.12    | <0.01 |
| Backcross stock   | 163                | 49.7                    | 24                | 1.54 | 57.12    | <0.01 |
| F <sub>1</sub> $\sigma^7 \times$ S $\phi$ $\phi$          | 29                 | 68.8                    | 5                 | 1.54 | 11.90    | <0.01 |
| F <sub>1</sub> $\phi$ $\phi \times$ S $\sigma^7 \sigma^7$ | 134                | 44.5                    | 19                | 1.49 | 41.37    | <0.01 |

If we examine the reciprocal crosses which made up the F<sub>1</sub> stock, there seems at first a noticeable difference in the reactions of the offspring. The  $R_1$  males appear to have transmitted more resistance to the young than did the  $R_1$  females in the reciprocal cross. This in itself is a clear case against any appreciable influence of passive immunity received from the dams, for within the knowledge of the author, there is no case reported in the literature wherein the male has transmitted passive immunity. The differences in favor of the higher resistance having been transmitted by the surviving males ( $R_1$ ) may well be attributed largely to the fact that these males used in the crosses were selected from those parents producing highly resistant litters, and the  $R_1$  females of the crosses were selected at random.

The third set of items in the table is concerned with the mortality of the backcrossed stock. Here we find that the F<sub>1</sub> males seemingly begot more susceptible offspring than the F<sub>1</sub> females, the reverse condition from the case above. Were this stock to be examined alone, it would present a convincing argument for the transmission of passive immunity by the surviving females to their offspring. However, the scarcity in the number of litters from the F<sub>1</sub> males may account in part for the differences observed.

It may be safely concluded, then, that in this experiment the effect of passive immunity has been at a minimum. Since the rats were inoculated at approximately three weeks after weaning, it would seem from that fact alone that any passive immunity would have practically disappeared before the time of injection.

#### THE INFLUENCE OF SEX ON RESISTANCE

During the early stages of this experiment, particularly while numbers of the organisms greater than the standard dose were being injected, it was noted that there was a scarcity of females among the survivors. This slightly greater surviving number of males was also observed in the S stock; in other stocks, however, the opposite condition was found as given in table 10.

TABLE 10  
*Percentage mortality by sexes of each of the different stocks.*

| STOCKS               | NUMBER<br>INJECTED | PERCENTAGE<br>MORTALITY | NUMBER<br>LITTERS | L    | $\chi^2$ | P     |
|----------------------|--------------------|-------------------------|-------------------|------|----------|-------|
| S stock              | 228                | 92.5                    | 46                | 1.49 | 101.66   | <0.05 |
| ♂♂                   | 130                | 90.0                    | 45                | 1.33 | 80.10    | <0.05 |
| ♀♀                   | 98                 | 95.9                    | 34                | 0.98 | 32.59    | 0.45  |
| Ra stock             | 431                | 84.7                    | 15 (groups)       | 1.15 | 19.77    | 0.14  |
| ♂♂                   | 237                | 82.7                    | 15 (groups)       | 0.98 | 14.37    | 0.43  |
| ♀♀                   | 194                | 87.1                    | 14 (groups)       | 0.95 | 12.55    | 0.36  |
| R <sub>1</sub> stock | 137                | 42.3                    | 23                | 1.60 | 58.80    | <0.05 |
| ♂♂                   | 67                 | 28.3                    | 22                | 1.01 | 22.75    | 0.36  |
| ♀♀                   | 70                 | 55.7                    | 20                | 1.42 | 40.60    | <0.05 |
| R <sub>2</sub> stock | 227                | 32.6                    | 33                | 1.38 | 63.03    | <0.05 |
| ♂♂                   | 103                | 35.9                    | 32                | 1.27 | 51.84    | <0.05 |
| ♀♀                   | 124                | 29.8                    | 33                | 1.20 | 47.85    | <0.05 |
| R <sub>3</sub> stock | 117                | 35.0                    | 18                | 1.66 | 49.84    | <0.05 |
| ♂♂                   | 45                 | 44.4                    | 18                | 1.07 | 20.56    | 0.15  |
| ♀♀                   | 72                 | 29.2                    | 18                | 1.47 | 39.06    | <0.05 |
| F <sub>1</sub> stock | 175                | 54.9                    | 32                | 1.75 | 97.90    | <0.05 |
| ♂♂                   | 89                 | 52.8                    | 28                | 1.44 | 58.24    | <0.05 |
| ♀♀                   | 86                 | 56.9                    | 31                | 1.38 | 59.27    | <0.05 |
| Backcross            | 163                | 49.7                    | 24                | 1.54 | 57.12    | <0.05 |
| ♂♂                   | 69                 | 50.7                    | 22                | 1.17 | 30.36    | 0.05  |
| ♀♀                   | 94                 | 48.9                    | 23                | 1.43 | 47.38    | <0.05 |
| F <sub>2</sub> stock | 200                | 25.5                    | 28                | 1.60 | 71.40    | <0.05 |
| ♂♂                   | 109                | 32.1                    | 27                | 1.46 | 57.78    | <0.05 |
| ♀♀                   | 91                 | 17.6                    | 27                | 1.23 | 40.90    | <0.05 |

There is here shown no uniform superiority throughout in the resistance of either sex. Were one to consider only the reactions of the S, Ra, and R<sub>1</sub> stocks, a very good argument for a higher resistance of the males could be offered. Particularly in the R<sub>1</sub> stock was there a distinct difference in mortality in favor of the males. The difference between the proportionate deaths of the sexes is significant in this case.

This same difference is not manifested for the remaining stocks of the table. With the exception of the F<sub>2</sub> stock, the differences in resistance between the sexes are no more than might be expected from random sampling for comparatively small numbers. The F<sub>2</sub> stock gave the opposite reaction to that of the R<sub>1</sub> stock, in that the females were more resistant. The lower number of proportionate deaths of the females of this stock as compared with the males is practically significant ( $P=0.02$ ). No explanation for the opposing reactions of the sexes of these two particular stocks can be offered as yet.

If each stock is considered in its reaction by sexes, it is worthy of note that the value of  $L$  for each sex is in general slightly lower than for the stock as a whole. This would suggest a slightly differential reaction between the sexes in resistance to this infection. The males in three of the stocks have shown a normal dispersion of mortality about their average in each of these stocks. This may in part be ascribed to the small numbers involved, and to unknown causes.

Mention has been made in this report that the S stock has shown a departure from what might be reasonably expected as a result of approximately sixty-two generations of brother-sister matings. If this stock is analyzed by sexes for a normal distribution of chances of death, the females showed a constant probability for death in the litters tested ( $P=0.45$ ). The reaction of the males gave no indication of an approach to the normal type of dispersion.

It was previously pointed out in this report that the chief discrepancies were found in four of the litters. It should be added that other litters of this S stock or other controls, injected on the same days as these four litters, deviated in no appreciable degree from the average mortality of the respective strains. By eliminating these four litters from the total, and analyzing the stock by sexes, the following is obtained:

|              | <i>Number<br/>injected</i> | <i>Percentage<br/>mortality</i> | <i>Number<br/>litters</i> | <i>L</i> | <i>X<sup>2</sup></i> | <i>P</i> |
|--------------|----------------------------|---------------------------------|---------------------------|----------|----------------------|----------|
| <i>Total</i> | 211                        | 96.2                            | 42                        | 1.37     | 78.9                 | <0.01    |
| ♂♂           | 119                        | 95.8                            | 41                        | 1.18     | 56.9                 | 0.05     |
| ♀♀           | 92                         | 96.7                            | 32                        | 0.97     | 30.4                 | 0.40     |

By a comparison with the S stock in table 10, these results indicate that the chief deviations from the average mortality were caused by the reactions of males within these four litters. It would seem, then, that litters within this inbred strain have differed somewhat in their reaction to this infection. Tests are in progress to determine whether these differences in resistance were hereditary or were due to outside causes.

If we consider the reactions of all the other stocks, the fact that neither the stocks selected for resistance nor the hybrid stocks have shown a uniform probability for death among the litters would in itself be a very good argument against taking the mortalities of such variable stocks as convincing in regard to differential resistance in favor of either sex. One stock only (Ra) has given indication of a constant underlying probability for death in the stock as a whole; and here also there has appeared a slight differential mortality between the sexes.

#### WEIGHT AS A FACTOR IN RESISTANCE

Since the ages of the animals at injection have varied but little for all stocks beyond the general average of 45-50 days, the average weights of the survivors and of those killed in the different stocks have been calculated as for a uniform age. The range in ages has been from 40-55 days; for this range, no appreciable advantage in resistance due to increased age has been noted.

TABLE 11

*Average weight in grams immediately preceding injection of survivors and non-survivors for each of the different stocks.*

| STOCK          | SURVIVORS | NON-SURVIVORS | DIFFERENCE | DIFF.<br>P.E. |
|----------------|-----------|---------------|------------|---------------|
| S              | 73.8±3.14 | 69.9±0.95     | 3.9±3.28   | 1.19          |
| R <sub>1</sub> | 89.0±1.63 | 78.5±1.34     | 10.5±2.11  | 4.97          |
| R <sub>2</sub> | 81.4±1.29 | 66.3±1.49     | 15.1±1.97  | 7.66          |
| R <sub>3</sub> | 78.6±1.25 | 60.0±1.57     | 18.6±2.01  | 9.25          |
| F <sub>1</sub> | 91.5±1.27 | 75.9±0.96     | 15.6±1.59  | 9.81          |
| Backcross      | 91.3±1.45 | 78.2±1.25     | 13.1±1.91  | 6.86          |
| F <sub>2</sub> | 73.6±0.89 | 64.7±1.38     | 8.9±1.40   | 6.36          |

The average weights for the survivors and non-survivors of practically all the stocks as given in table 11 would seem to indicate at first glance that the heavier individuals have had a considerable advantage in resistance. However, certain limiting factors should be considered before a general conclusion may be drawn that the heavier, and presumably the more vigorous, animals are favored in resistance to this infection.



The stocks which showed the significant advantages for resistance among the heavier animals were quite heterogeneous in hereditary factors for resistance, judging from the value of  $P$  for each in table 8. This lack of uniformity was manifested in each stock in the number, and also in the weights, of the individuals within the litters.

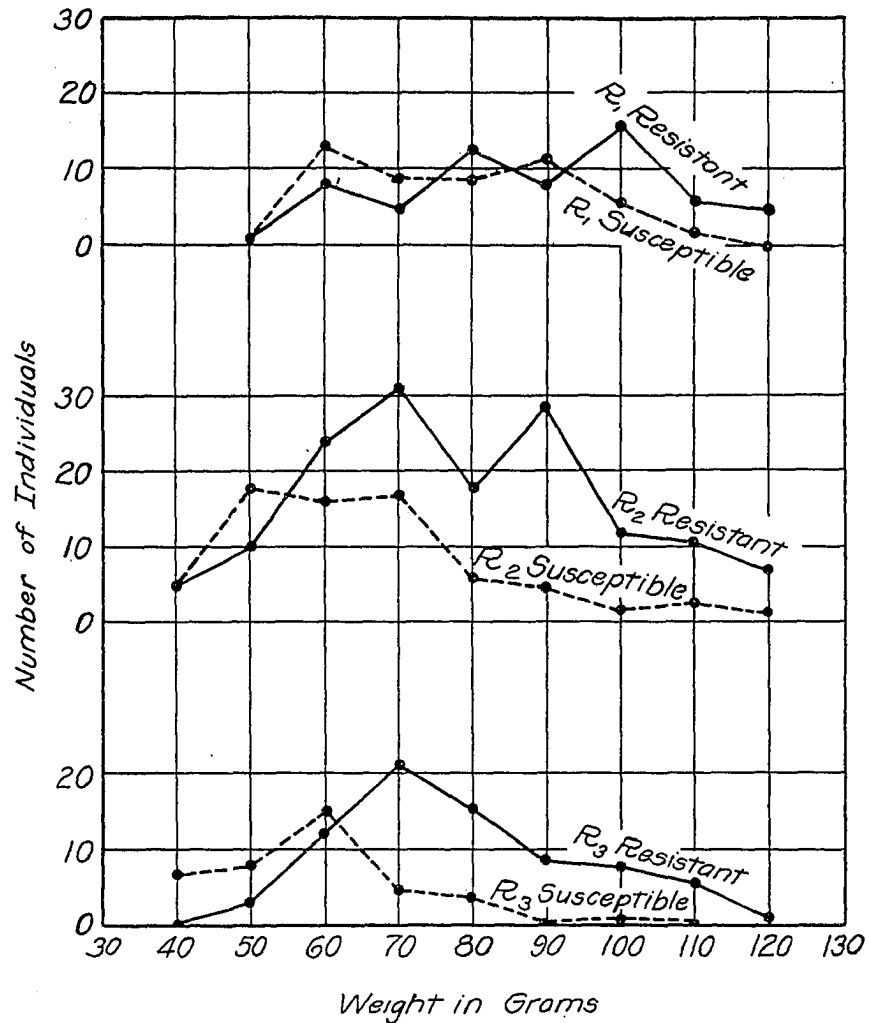


FIGURE 4.—A comparison of the weights of the resistant and susceptible individuals in stocks selected for resistance.

Progressive differences in the weights between survivors and non-survivors are recorded for the successive resistant stocks. In figure 4 a comparison is given between the frequencies of the different weights for those resistant (survivors) and susceptible (non-survivors) of these selected

stocks. From this graph it may be seen that, particularly in the  $R_2$  and  $R_3$  stocks, there is a trend towards more resistance in the heavier individuals, and conversely that the smaller individuals are more inclined to be susceptible to the disease.

Were weight in itself a major factor in resistance to this infection, it would logically follow that the survivors of a susceptible strain would be among those of the greatest weight at a given age. The differences in weight between the survivors and non-survivors of the susceptible S stock hardly bear out this assumption. The survivors of this stock were not necessarily the heaviest, although their average weight was slightly higher than that of the non-survivors.

If the reaction of this susceptible stock is of value in determining the influence of weight on resistance, it can hardly be argued that increased weight in itself is always associated with increased resistance. The litters of the S stock varied in weight from litter to litter as did the other stocks, largely due to differences in the number within the litters, but were quite uniform in weight within each litter. It cannot then be maintained that they were less capable of growth than the other-stocks.

The data as presented are indicative that the individuals in stocks other than the S, which possessed greater weight also possessed somewhat greater resistance; but they are far from conclusive. Greater weight in itself has not been shown to be a major determining factor in resistance to this infection. Whether the advantage in resistance of the heavier individuals was due to an association of the factors for growth with the factors for resistance, cannot be determined accurately as yet. Conclusive evidence concerning the effect of weight on resistance should await the production of stocks more uniform in their reaction towards this infection.

#### THE RESULTS OF INBREEDING

Many litters within the  $R_2$  and particularly within the  $R_3$  stock, resulting from brother-sister and other closely related matings, were characterized by a distinctly higher mortality than the average of either stock. Also there was a slight trend for many of these litters to be less in weight than other litters of the same age. These smaller individuals, upon injection, generally produced a somewhat higher mortality than those of larger size, although the parentages of many litters differing considerably in size were the same.

To test the influence of the higher mortality of these smaller individuals on the general average of the  $R_2$  and  $R_3$  generations, each stock was divided into two classes according to weight, with 65 grams as the arbi-

trary dividing line, and the mortality of each class was calculated as follows:

| <i>Stock</i>           | <i>Number<br/>injected</i> | <i>Percentage<br/>mortality</i> |
|------------------------|----------------------------|---------------------------------|
| R <sub>2</sub> (total) | 227                        | 32.6                            |
| Above 65 grams         | 149                        | 23.5                            |
| Below 65 grams         | 78                         | 50.0                            |
| R <sub>3</sub> (total) | 117                        | 35.0                            |
| Above 65 grams         | 72                         | 15.3                            |
| Below 65 grams         | 45                         | 66.7                            |

There is here shown a tendency for the susceptible animals to be less in weight than those resisting the disease. This effect is greater in the R<sub>3</sub> than in the R<sub>2</sub> stock, and may be due indirectly to the results of inbreeding. The same general tendency is shown in figure 4 assuming that the increase in number of smaller individuals in the R<sub>2</sub> and R<sub>3</sub> stocks, respectively, over each parent stock, was due in part to the effects of inbreeding.

It might well be argued from these results that if we attribute the small size of the individuals in many of the litters within these stocks to a concentration of many factors unfavorable for growth, as a result of inbreeding, factors unfavorable for resistance were also brought together in many of these cases. It can hardly be maintained that the factors for normal growth in these stocks were the same as the factors for resistance, for within the susceptible S stock, many individuals of more than average weight seemingly lacked factors for resistance. Further data are necessary, however, before much more than a suggestion can be made in this connection.

#### A COMPARISON OF THE INFLUENCE OF CERTAIN MALES

An ideal experiment which would accurately measure any possible fluctuations in the ability of a sire to transmit resistance to his offspring would necessitate that all the females used in mating should have the same genotypic potentiality for resistance. Obviously for an experiment of this nature, and at its present stage, this criterion has been an impossibility. There have been noted, however, certain differences in the reactions of the progenies of different sires used in this experiment that make them worthy of comparison.

To give a somewhat similar basis for a comparison of various sires, only those for which the same general degree of relationship has existed between the males and females of the matings have been presented. An exception to this is found in the matings of the males of the surviving Ra stock, which were quite at random.

Part A of table 12 is concerned with the reactions of the progenies of

different males of the Ra stock which had survived the infection. With but one exception, the values for L of these progenies, with the resulting values of P, indicate that the females with which they were mated differed not greatly from themselves in ability to transmit resistance to their offspring. The tests for independence between these males as given in table 13, are good indications that marked differences in potential resistance were present in survivors of a fixed dose of the organism, as evidenced by the resistance of their offspring to the same fixed dose.

Further evidence on this point is given in the reactions of the progenies of the two males, 10.3 and 11.1 (Part B). These two males were the only survivors of seven R<sub>1</sub> individuals injected with approximately twice the standard dose. The male 11.1 evidently possessed much more ability to transmit resistance to his offspring than did the other; even for these small numbers, the difference in proportionate deaths of the progeny of these two males is significant.

Of the remaining five males listed in part B, four are full brothers;

TABLE 12

*A comparison of the mortality of the progenies of certain sires selected for resistance.*

| SIRES  | NUMBER<br>INJECTED | PERCENTAGE<br>MORTALITY | NUMBER<br>LITTERS | NUMBER<br>DIFFERENT<br>DAMS | L    | $\chi^2$ | P     |
|--|--------------------|-------------------------|-------------------|-----------------------------|------|----------|-------|
| Part A. R <sub>1</sub> generation              |                    |                         |                   |                             |      |          |       |
| Ra4  | 52                 | 19.2                    | 8                 | 3                           | 1.29 | 13.44    | 0.06  |
| Ra18   | 40                 | 40.0                    | 7                 | 4                           | 1.27 | 11.27    | 0.08  |
| Ra28   | 18                 | 50.0                    | 3                 | 2                           | 1.10 | 3.63     | 0.17  |
| Ra32   | 14                 | 85.7                    | 3                 | 2                           | 1.51 | 6.87     | 0.03  |
| Part B. R <sub>2</sub> generation              |                    |                         |                   |                             |      |          |       |
| 10.3   | 10                 | 90.0                    | 2                 | 1                           | 0.74 | 1.11     | 0.29  |
| 11.1   | 30                 | 16.7                    | 4                 | 3                           | 0.77 | 2.37     | 0.50  |
| 1.1  | 44                 | 36.4                    | 6                 | 4                           | 1.05 | 6.66     | 0.25  |
| 5.2  | 16                 | 18.8                    | 3                 | 2                           | 1.25 | 3.75     | 0.16  |
| 5.4  | 19                 | 10.6                    | 4                 | 2                           | 0.76 | 2.32     | 0.50  |
| 36.1   | 70                 | 44.2                    | 7                 | 7                           | 1.50 | 15.75    | <0.01 |
| 36.2   | 32                 | 43.7                    | 7                 | 4                           | 1.26 | 6.38     | 0.09  |
| Part C. Backcrossed generation                 |                    |                         |                   |                             |      |          |       |
| 1.1  | 31                 | 51.6                    | 6                 | 3                           | 1.08 | 6.48     | 0.26  |
| 1.2  | 16                 | 62.5                    | 6                 | 3                           | 1.06 | 6.78     | 0.41  |
| Part D. R <sub>3</sub> generation <sup>1</sup> |                    |                         |                   |                             |      |          |       |
| 25.1   | 39                 | 79.5                    | 7                 | 7                           | 1.14 | 9.07     | 0.18  |
| 18.1   | 20                 | 60.0                    | 2                 | 2                           | 0.82 | 1.33     | 0.25  |
| 18.2   | 23                 | 47.8                    | 4                 | 4                           | 1.57 | 9.91     | <0.01 |
| 19.1   | 25                 | 16.0                    | 3                 | 3                           | 1.07 | 3.42     | 0.18  |
| 19.3   | 23                 | 17.4                    | 3                 | 2                           | 0.54 | 0.88     | 0.83  |

<sup>1</sup> The mortalities of several litters not given in former tables are included here.

all five in fact were descendants of the male Ra4. Each of the five was mated to certain of his full sisters; three were outcrossed to females quite closely related. No appreciable differences in mortality were found in the progeny from either type of mating for these particular males.

TABLE 13  
*Test for independence between the offspring of certain sires given in table 12.*

| SIREs<br>COMPARED | DIFFERENCES IN<br>MORTALITY | $\chi^2$ | P     |
|-------------------|-----------------------------|----------|-------|
| Ra4 and Ra18      | 30.8                        | 4.82     | 0.03  |
| Ra4 and Ra28      | 40.8                        | 6.35     | 0.01  |
| Ra4 and Ra32      | 66.5                        | 25.89    | <0.01 |
| Ra18 and Ra32     | 45.7                        | 11.73    | <0.01 |
| 10.3 and 11.1     | 73.3                        | 17.73    | <0.01 |
| 5.4 and 36.1      | 23.6                        | 7.18     | <0.01 |
| 19.1 and 25.1     | 63.5                        | 24.40    | <0.01 |
| 18.1 and 19.1     | 44.0                        | 9.35     | <0.01 |

The male 1.1 produced progeny quite uniform<sup>1</sup> in resistance for the different litters. When mated to susceptible S females, as given in C, a similar uniform distribution of chances for death of the progeny was found, with a somewhat higher mortality. A litter brother, 1.2, when similarly mated, seemed also quite uniform in his ability to transmit resistance to his offspring.

The other males of this  $R_1$  generation, full brothers but from two litters, differed considerably in their ability to transmit resistance, judging from the reaction of their progenies. The offspring of each male, with the exception of that of 36.1, had no dispersions from their average mortalities not well within the limits of chance.

Differences in the proportion of deaths for the offspring of males 5.4 and 36.1 indicate a very wide difference in the concentration of potentialities for resistance in each sire ( $P < 0.01$ , table 13). This is true also in general for 5.4 when compared with 36.2; and to a less extent, perhaps, for the advantage in the ability of 5.2 to transmit resistance when compared with that of either 36.1 or 36.2.

The progeny of 5.4 produced a very constant distribution of chances for death, varying slightly from litter to litter ( $P = 0.50$ ). These litters were the result of only brother-sister matings, and it would seem that they, in turn, should pass on this immunity to their respective offspring.

The male 25.1 (D) is a result of such a mating of 5.4. The  $R_3$  progeny of this male, from matings to sisters or to closely related females, showed a decided drop in the proportionate number of resistant individuals

from that of the immediate parents. Unfortunately, this is the only male resulting from brother-sister matings involving the male 5.4 above, whose progeny records are available at present. We can therefore make no comparison for a similar increase in mortality of progeny from litter brothers of the male 25.1.

However, if we compare the progenies of males 18.1 and 18.2 with those of 19.1 and 19.3, all of which males were produced from two brother-sister matings involving the male 1.1, we find considerable difference in mortality. Here again, either brother-sister matings or matings to closely related females were made for each of the four males. The progenies of the males, 19.1 and 19.3, were much more resistant than those of 18.1 and 18.2, as shown most clearly by the difference in proportionate deaths between the offspring of 19.1 and 18.1 ( $P < 0.01$ ).

Inbreeding in the case of these five  $R_2$  males seemingly has resulted in a considerable difference in the concentration of factors making for resistance in each. With the exception of 18.2, the values of L and P for the reactions by litters of the progenies indicate that each male was mated to females of approximately the same potentiality of resistance.

The reactions of the progenies of the several males given in the table offer a strong indication that the factors for resistance possessed by these individuals were in large part the factors of heredity. It has been shown that certain animals surviving an infection differed appreciably in their ability to transmit this same resisting power to their offspring. We have good grounds for postulating, then, that the survivors which transmitted resistance to but a small proportion of their offspring were heterozygous themselves for resistance factors; they were the result of a chance combination of factors (complementary in nature perhaps) for resistance, and their offspring depended for their survival upon the chance recombination of factors making for resistance.

The survivors whose progeny were quite highly resistant were either relatively homozygous for resistance factors, or possibly each of the matings resulted in a fortunate combination of these factors.

The possible effects of inbreeding on resistance have been well demonstrated in the mortalities of the progenies of these different males of the  $R_2$  stock. Results up to the present have shown that inbreeding may result in a concentration of hereditary factors either for resistance or for susceptibility.

#### SUMMARY

Differences in host resistance to the injection of a fixed dose of a specific organism have formed the basis of these investigations. The organism

used throughout, a member of the paratyphoid-enteritidis group of bacteria, has shown no measurable difference in virulence over a period of 18 months. The results that have been presented are based on intraperitoneal injections of over 2000 rats as hosts.

A stock of rats, descendants of the Wistar A strain, inbred brother-sister for approximately sixty generations, has proven highly susceptible (92.5 percent mortality) to the fixed dose of the organism. Differences in resistance within this line have been noted, however.

The progeny of each of three successive generations of survivors of a pen inbred stock to a fixed dose of the organism has shown a definite increase in the number of individuals resistant to this infection when compared with members of the same stock not so selected.

There was no indication that the increased resistance of the selected stock was due to the transmission of passive immunity.

Preliminary crosses were made to determine whether or not the differences between the first resistant generation (42.3 percent mean mortality) and the susceptible stock (92.5 percent mean mortality) could be attributed to hereditary factors. The  $F_1$  progeny of this cross showed a somewhat closer approach in mean mortality to that of the resistant parent than to that of the susceptible parent, being 54.9 percent. Matings of the survivors of this  $F_1$  stock to the susceptible strain produced the backcrossed progeny, with 49.7 percent mean mortality: the progeny ( $F_2$ ) of matings of the  $F_1$  stock survivors among themselves gave 25.5 percent mean mortality. Were it not for the fact that the parental stocks of these hybrid generations were themselves heterozygous for resistance factors, the reactions of the backcrossed and  $F_2$  stocks would indicate a single factor difference for resistance and susceptibility to this infection. This is hardly possible for there was no normal dispersion of chances for death within these hybrid stocks.

Certain stocks have shown a differential action in resistance between the sexes, unexplainable at present. No constant advantage in resistance for either sex has been found.

The heavier individuals of the selected and hybrid stocks were somewhat more resistant than those of less weight. This advantage of increased weight on resistance was not manifested within the susceptible stock, however.

A slight decrease in size which accompanied inbreeding within the resistant stocks was also somewhat closely associated with decrease in resistance.

Distinct differences between individuals in their ability to transmit resistance to their offspring have been noted.

It is suggested that the resistance of this host to the specific organism is in large part dependent upon the action of a complex of hereditary factors, some of which are at least partially dominant.

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